# PRELIMINARY NOTE ON A TOXIN-PRODUCING ANAEROBE ISOLATED FROM THE LARVÆ OF LUCILIA CÆSAR.

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Certain material was recently received at the Hygienic Laboratory from Dr. E. W. Saunders, to be tested for the presence of an "unknown pathogenic microbe" believed by the sender to be concerned in limberneck of chickens.[1] The results of the work on this material are of interest in that the presence of an anaerobic organism producing a soluble toxin has been demonstrated, which, in its effects on animals, behaves in a manner similar to that of the toxin of the organism of botulism, but which, however, fails to be neutralized by the antitoxins of either Type A or B of *Clostridium botulinum*.

The material as received at various times consisted of the carcasses of one guinea pig and three chickens and a collection of the larvæ of the green fly *Lucilia cæsar* preserved in glycerin.

The material was cultured liberally in meat mash media and glucose broth fermentation tubes. Extracts of certain portions of the material and cultures 8 or 9 days old of the material were found to be very toxic to mice when inoculated intraperitoneally according to the technique recently suggested by the author for testing suspicious foods for the presence of botulinus toxin (Pub. Health Rep., 1921, *36*, 1665. Reprint No. 677.) All such cultures and extracts consistently failed to be neutralized by polyvalent botulinus antitoxin.

The organism was isolated by means of agar shake cultures, various types of colonies being fished to meat mash media tubes, which, after a period of incubation, were tested on mice as before. The particular culture with which this work was done was isolated from a tube planted with one of the larvæ preserved in glycerin. The culture was passed through several meat mash media and glucose agar shake cultures, single colonies being fished each time from the glucose agar tubes.

The most striking cultural characteristics of the organism may be described as follows: It is apparently nonproteolytic in meat mash media, in which it grows without, as a rule, producing any marked turbidity or change in the appearance of the meat. A large amount of gas is produced, bubbles continuing to form for long periods (7 days or longer). The gas bubbles sometimes are the only evidence of growth. In contrast to this behavior in meat media, no production of gas has been observed in glucose broth or in glucose agar shake cultures. The appearance of growth in broth media is characteristic. Instead of a homogeneous appearance as is obtained with

[1] Saunders, E. W., Wisdom, W. E., and White, T. W., The *Lucilia cæsar* Epizootic, Transmitted Through its Toxivirulent Larvæ, and its Relation to Simian and Human Poliomyelitis. Jour. Missouri State Med. Assoc., 1921, *18*, 4.

the organism of tetanus and botulinus, a flaky growth occurs, the organisms apparently being agglutinated and in the course of several days being deposited on the sides and at the bottom of the tube. The appearance of colonies in deep glucose agar cultures is in marked contrast to the solid lenticular colonies of *Clostridium botulinum*. They are very fluffy and without a compact central nucleus.

The following is a more detailed record of the cultural and morphological characteristics.

*Morphology and staining properties.*—In smears made from 24-hour-old cultures the organism appears as a rod with rounded ends, which occurs usually singly, but sometimes in pairs and short chains. The rods are often slightly curved. The size is about 3 to 6 by 0.5 to 0.8 $\mu$.

The organism is gram-positive in young cultures, but in older cultures, gram-positive individuals are rare.

Spores appear in meat media in 48 hours and after a longer period in $\frac{1}{10}$ per cent agar cultures. The spores are terminal and somewhat wider than the rod. The number of spores was comparatively few in all of the smears examined.

*Motility.*—Hanging drop preparations made from 24-hour cultures in glucose broth, meat media, and $\frac{1}{10}$ per cent agar showed nonmotile organisms. The usual technique was followed and no precautions were taken to exclude oxygen.

*Cultural requirements.*—The organism requires anaerobic conditions in media not containing meat. In agar stab and glucose agar shake cultures the growth extends from the bottom of the tube to about 1 cm. below the surface of the medium. The boiling of meat media previous to inoculation serves to expel the air, and no further precautions to secure anaerobiosis are required.

A temperature of 37.5° C. is favorable for growth; but growth is also obtained after a period of delay, in meat media held at room temperature.

On the whole, the organism grows less readily than the strains of *Clostridium botulinum* isolated in this country. Single colonies fished from glucose agar media often fail to grow if cultures are much over one week old; and occasionally when conditions are apparently favorable, no growth is obtained. The appearance of growth in various media is usually delayed until the second day of incubation.

*Cultural characteristics.*—Agar stab cultures: A rather scant growth appears along the line of needle puncture with no evidence of gas formation.

One-tenth per cent agar medium: This medium is a favorable one for the growth of the organism, a fairly heavy growth developing in 24 to 48 hours.

Glucose agar shake cultures: The colonies, as stated above, are of the fluffy type. Although the tubes may be crowded with colonies, no gas bubbles have been observed.

Liver agar shake cultures: The colonies in this medium at first resembled the typical lenticular colonies of *Clostridium botulinum*, but later became fluffy. Gas bubbles were present in tubes containing a moderate number of colonies.

Gelatin: Scant growth has been obtained in gelatin stab cultures, with no liquefaction in 14 days.

Litmus milk: An acid reaction is produced in milk after an incubation period of 48 hours. No coagulation or digestion of casein occurred in 14 days.

Meat mash media: The meat mash medium consists of one part of chopped meat to two parts of distilled water, adjusted to a reaction of $p_H$ 8 and autoclaved at 15 pounds pressure for 1½ hours. Growth occurs quite readily in this medium, with the development of only slight turbidity and numerous gas bubbles.

Fermentation reactions: Growth occurs in glucose broth in the course of 48 hours, with slight acid production, but without formation of gas. No growth was obtained in lactose and saccharose broth.

Good growth was obtained in liver broth, often with the formation of gas.

*Thermal death point.*—This has not been determined accurately, but a few tests were carried out to determine roughly the temperature and length of time required to destroy the spores. Seven-day-old cultures were heated in the Arnold sterilizer for one-half hour and one hour (temperature 93 to 95° C.). Growth was obtained in tubes of meat media planted with the tube heated for one-half hour, but none with that heated for one hour.

*Toxin.*—Toxin was produced in meat media cultures after two or three days, or after longer periods of incubation Two-tenths c. c. of the toxic filtrate inoculated intraperitoneally was usually fatal to mice within 5 or 6 hours, and smaller amounts in a correspondingly longer time. The toxin was tested on animals by subcutaneous and intraperitoneal inoculations and by feeding. The results are presented in detail below.

A few tests were made which gave some information in regard to the temperature necessary to destroy the toxin. Animals inoculated with a dose of 0.01 c. c. of filtrate heated to 60 to 65° C. for 20 minutes developed no symptoms, the untreated filtrate being toxic in doses of 0.001 c. c. or less. Toxin heated to 60° C. for 10 minutes only was toxic for both guinea pigs and rabbits, though death was somewhat delayed as compared with the results of tests in animals receiving unheated toxin.

Tests on animals: A number of parallel tests were carried out on animals, with cultures and the corresponding filtrates. In most cases similar results were obtained, with both, the time of death of the animals inoculated with filtrates being somewhat delayed as compared with the time of death of those receiving cultures. The results presented are those obtained with filtrates unless otherwise specified.

*Guinea pigs.*

| Amount and method of administering toxin: | Time elapsing before death. |
|---|---|
| 0.001 c. c. inoculated subcutaneously | hours.. +29 |
| 1 c. c. inoculated subcutaneously | do.... + 6 |
| 1 c. c. fed | do.... +29 |
| 0.1 c. c. fed | Survived. |
| 0.1 c. c culture fed | hours.. +16 |

*Rabbits.*

| | |
|---|---|
| 0.001 c. c. inoculated subcutaneously | hours.. +40 |
| 1 c. c. inoculated subcutaneously | do.... +16 |
| 1 c. c. fed | Survived. |
| 1 c. c. culture fed | Survived. |

*Rats.*

| | |
|---|---|
| 0.001 c. c. inoculated subcutaneously | hours.. +28 |
| 0.01 c. c. inoculated subcutaneously | do.... +16 |
| 1 c. c. inoculated subcutaneously | do.... + 5 |

*Mice.*

| | |
|---|---|
| 0.001 c c. inoculated intraperitoneally | hours.. + 9 |
| 0.1 c. c. inoculated intraperitoneally | do.... + 6 |

*Monkeys.*

| | |
|---|---|
| 0.01 c. c. inoculated subcutaneously | hours.. +71 |
| 4-5 g. of meat culture fed on bread | do.... +71 |

*Pigeons.*

| | |
|---|---|
| 1 c. c. of culture fed | hours.. +31 |
| 1 c. c. of culture fed | days.. + 7 |

*Chickens.*

| | |
|---|---|
| 1 c. c. inoculated subcutaneously | Survived. |
| 5 c. c. of filtrate fed | No symptoms in 72 hours. |
| 4-5 g. of meat culture fed on bread | Developed symptoms in 24 hours but recovered later. |

Tests were made on guinea pigs to determine whether any protection was afforded by several different antitoxins against small amounts of the toxin, as follows:

| Amount of filtrate. | Antitoxin. | Potency. | Symptoms. | Time elapsing before death. |
|---|---|---|---|---|
| 0.0001 c. c. | 1 c. c. polyvalent botulinus antitoxin. | Over 2,500 units Type A; 75 units Type B. | 6-7 days... | + 12 days. |
| Do. | 1 c. c. type B botulinus antitoxin. | 140 units | ...do..... | + 10 days. |
| Do. | 1 c. c. serum from cow 2770.[1] | | ...do..... | + 17 days. |
| Do. | No antitoxin | | ...do..... | + 16 days. |

[1] Serum received from Dr. Robert Graham and obtained by immunizing a cow against an organism also obtained from limber neck material.

All of the above animals died in from 10 to 17 days and all had exhibited symptoms of hypotonicity and emaciation to about the same degree in 6 or 7 days.

The results of another test are as follows:

| Amount of filtrate. | Antitoxin. | Potency. | Symptoms. | Time of death. |
|---|---|---|---|---|
| *c. c.* | | | *Hours.* | *Hours.* |
| 0.001 | 1 c. c. polyvalent botulinus antitoxin. | Over 2,500 units Type A; 75 units Type B. | 43 | +44 |
| Do | 1 c. c. Type B botulinus antitoxin. | 140 units | 43 | +45 |
| Do | 1 c. c. serum from cow 2770 | | 43 | +47 |
| Do | 1 c. c. normal horse serum | | 43 | +47 |
| Do | No antitoxin | | 27 | +42 |

The results of this test indicate that some slight protection may have been afforded by all of the serums used in that the animal which received no serum died several hours earlier than the others, but the differences are not great enough to be significant. In comparison with normal horse serum, no protection was afforded by any of the antitoxins used.

These tests and others not included therefore show that the filtrate of the organism isolated is toxic on inoculation and also by mouth to certain animals, as is the toxin of *Clostridium botulinum*, but that no protection is afforded by polyvalent botulinus antitoxin in inoculation tests.

As to the specific effects produced on laboratory animals, the symptoms closely resemble those of botulism and include general hypotonicity of muscles, increased salivation, and prostration. Guinea pigs showing severe symptoms lie flat on the abdomen with head outstretched and are unable to stand. Rabbits show prostration and assume a crouched attitude, with the appearance of being unable to support their weight on the legs. When an attempt is made to run, there appears to be difficulty in coordination of the leg muscles. Chickens and pigeons exhibited closed or partially closed eyelids. The most noticeable symptoms in these animals was the inability to stand. There was some tendency to keep the head down; but on the whole, the effects produced on the limbs were most pronounced. Monkeys are inactive and present the appearance of illness, with increased flow of saliva from the mouth, ptosis of eyelids, and prostration.

*Pathology.*—Animals inoculated with cultures of the organism show some congestion at the site of inoculation, but very slight or no congestion when filtrates are inoculated. The liver may present a hyperemic appearance, and there is sometimes congestion of the upper intestine. Congestion of the adrenals has been noted in guinea pigs. The most striking feature, as in botulism, is in the marked

congested condition of the blood vessels of the brain and meninges. Sections of the organs have not as yet been examined for the occurrence of thrombi.

*Immunity.*—Rabbits are being inoculated with small increasing amounts of toxin to determine whether an antitoxin can be produced. Two methods are being pursued in this work; one being the inoculation of very small nonlethal doses of filtrate and the other the inoculation of larger doses of heated toxin.

COMMENT.

This study indicates that the organism described varies markedly from the strains of *Clostridium botulinum* isolated in the United States. Culturally and immunologically it appears to be a rather distinct organism. One is almost tempted to consider it as more closely related to the type originally described by von Ermengem,[4] when its nonproteolytic behavior and apparently low thermal death point are taken into consideration. There are, however, several important differences between the two organisms. The absence of gas production in glucose beef infusion media is noteworthy. Von Ermengem describes rich gas formation in glucose agar stab cultures and describes the medium as torn and fragmented. Gas formation in glucose broth is also emphasized. No mention is made of the flaky appearance of the growth in glucose broth. This is so distinctive that it seems improbable that it should have escaped notice if it had been present. Litmus milk is described as not being changed or coagulated. The organism under discussion produces a definitely acid reaction in litmus milk. The odor of the organism of von Ermengem is described as a penetrating, butyric-acid-like odor. There is no noticeable odor in cultures of the organism being studied. The colony formation in deep glucose agar tubes is not definitely described by von Ermengem. There is, however, no question as to the very diverse appearance in this medium of the colonies of the organism in hand and the colonies of cultures of *Clostridium botulinum* described in this country. The colonies in liver media, on the other hand, resemble in their early development the colonies which have been described as typical for the botulism organism. Von Ermengem's organism was found to be only slightly toxic to rats on inoculation. This organism is as toxic to rats as to some of the other animals tested.

Regarding the relation of the organism to limber neck in chickens, it can not be definitely stated at this time that it is etiologically concerned. The results obtained so far in experimental work have not been as promising as had been expected. Relatively large doses seem to be required to produce symptoms. It is possible, however,

[4] Von Ermengem, Der Bacillus botulinus und der Botulismus (*in* Kolle u. Wassermann, Handbuch der pathogenen Mikroorganismen, 1912, *4*, 909–938.

that the age of the fowl, the breed, and other factors may have a bearing on the results, and further work is needed along this line as well as on the relation of the fly *Lucilia cæsar* to the disease.

## COURT DECISION ON PURIFICATION OF WATER SUPPLY.

The Court of Appeals of Kentucky has decided [1] that under the statutes the State board of health can forbid the furnishing by a company of impure water to a community on the ground that such water is a nuisance, but can not direct the use of any particular method of purification. The following is a portion of the court's opinion:

* * * We have no doubt that the State board of health may, under said section 2057, abate any nuisance in this State caused by filth which induces sickness. In this respect the powers of the board are broad, but not unlimited, and must be exercised within a sound discretion; not whimsically or capriciously nor arbitrarily. If the board of health in dealing with such matters does not exceed its powers nor abuse its discretion, its orders will be upheld by courts as final and conclusive. * * *

Although, as said above, the board of health has the power to abate a nuisance, source of filth or cause of sickness, it has no mandatory power enabling it to direct the method by which the result shall be accomplished. It can only cause the abatement of the nuisance, and is not concerned with the method by which it is done. In other words, it may stop the furnishing of impure and dangerous water to a community, but it has no power to direct a water company to install any particular character of plant for sedimentation, filtration, or chlorination of the water, and the water company may adopt any system that may seem best or expedient to it, if the system adopted produces the results desired—clear, soft, wholesome water. * * *

## DEATHS DURING WEEK ENDED JAN. 14, 1922.

*Summary of information received by telegraph from industrial insurance companies for week ended Jan. 14, 1922, and corresponding week, 1921. (From the Weekly Health Index, Jan. 17, 1922, issued by the Bureau of the Census, Department of Commerce.)*

| | Week ended Jan. 14, 1922. | Corresponding week, 1921. |
|---|---|---|
| Policies in force | 48,548,844 | 45,700,065 |
| Number of death claims | 10,240 | 9,697 |
| Death claims per 1,000 policies in force, annual rate | 11.0 | 11.1 |

[1] Prunell et al. *v.* Maysville Water Co., 234 S. W., 967.